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Advanced Materials to Enhance Central Nervous System Tissue Modeling and Cell Therapy

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Abstract

The progressively deeper understanding of mechanisms underlying stem cell fate decisions has enabled parallel advances in basic biology—such as the generation of organoid models that can further one's basic understanding of human development and disease—and in clinical translation —including stem cell based therapies to treat human disease. Both of these applications rely on tight control of the stem cell microenvironment to properly modulate cell fate, and materials that can be engineered to interface with cells in a controlled and tunable manner have therefore emerged as valuable tools for guiding stem cell growth and differentiation. With a focus on the central nervous system (CNS), a broad range of material solutions that have been engineered to overcome various hurdles in constructing advanced organoid models and developing effective stem cell therapeutics is reviewed. Finally, regulatory aspects of combined material-cell approaches for CNS therapies are considered.

Keywords

central nervous system; materials; organoids; stem cell; therapeutics

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Conflict of Interest

D.V.S. and R.J.M. are inventors on patents related to stem cell manufacturing, and D.V.S., R.J.M., R.G.S., and H.J.J. are co-founders of a company that works on stem cell manufacturing.

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1. Introduction

The central nervous system (CNS) is the region of the nervous system responsible for integrating sensory stimuli, processing this information, and executing a response. It consists of two main components: the brain, which is responsible for information processing and body function coordination both consciously and unconsciously, and the spinal cord, which serves as a conduit for transmitting the signals from the brain to the peripheral system and for controlling certain musculoskeletal reflexes independently from the brain. The two main types of tissues in the CNS are classified as gray matter—consisting of nerve cell bodies, dendrites, and axons, and white matter—consisting primarily of myelinated axons.[1]

At a high level, throughout embryonic development stem cells arising from the fertilized egg divide, specialize, and self-organize to give rise to a full, complex organism including the CNS. During the first two weeks of development, the process of gastrulation gives rise to the three germinal layers: ectoderm, mesoderm, and endoderm. The ectoderm—the origin of the CNS—subspecializes to form the neuroectoderm, which in turn gives rise to the neural crest and neural tube. The latter is composed by neuroepithelial cells, which are the early neural stem cells (NSCs), that initially divide symmetrically to expand their population, and later asymmetrically to generate multipotent progenitors, differentiated neurons, and glial cells.[2] The region of the neural tube in which neurogenesis occurs is termed the ventricular zone (VZ). This transient embryonic layer of tissue contains NSCs that line the ventricular system, which contains the cerebrospinal fluid (CSF). In addition, the embryonic ventricular system contains growth factors and other nutrients needed for neurogenesis. Newborn neurons progressively migrate outward from the VZ, leading to thickening of the tissue and ultimately the formation of the brain and spinal cord.

Developing a deep understanding of the mechanisms underlying CNS formation, as well as ways in which this tissue can fail, would offer insights into basic human development as well as approaches for cell replacement therapies (CRTs) to treat human disease and injury. However, a major limitation for such efforts is the lack of suitable models that recapitulate the complex environment of human neural tissue. Animal models for in vivo modeling have been widely adopted for studying these diseases;[3,4] however, these models often fail to fully recapitulate human disease due to fundamental biological differences between species. Fortunately, pluripotent stem cells (PSCs)—which have the capacity to generate all cell types of the adult body, including the CNS—offer great potential for emulating and investigating human CNS development and disease, as well as for generating desired neural subtypes with potential for treating neurological conditions. Furthermore, the advent of induced pluripotent stem cells (iPSCs), which can be derived from nearly any somatic cell such as skin or blood, enables the generation of patient “personalized” models to study genetic influences on human disease.[5]

In addition to their application to modeling CNS development and disease, stem cells are promising candidates to treat CNS disorders—such as neurodegenerative diseases, traumatic brain or spinal cord injuries, or stroke—which remain significant clinical challenges worldwide.[6] In general, the endogenous capacity of the CNS to regenerate is highly

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limited, and pharmacological and other interventions for such disorders largely address symptoms without modifying disease progression.[7–9] Therefore, the use of stem cells for cell-replacement therapies offers the unique opportunity to repair the damaged tissue and thereby potentially restore motor and/or cognitive functions. In particular, cortical neurons,[10] cortical interneurons,[11] medium spiny neurons,[12] oligodendrocyte precursors,[13] and dopaminergic neurons[14] have been generated by differentiating human stem cells in culture and offer potential to treat neurological conditions.

The potential of stem cells across the diverse applications described above is promising, yet one overarching challenge is identifying the optimal cellular microenvironmental conditions to instruct specific cellular outcomes, such as high yield expansion, lineage-specific differentiation, robust integration and functionality, and ultimately rebuilding complex multicellular tissues when implanted in vivo for therapeutic use. To overcome these challenges, materials can be engineered to interface with cells in specific ways to promote desired cell outcomes. Here, we review novel advanced materials, defined as materials engineered in the last decade to possess advantageous features in cell culture, including reproducibility and tunable biochemical and biophysical properties, that can be utilized to guide cell fate and improve efforts across the spectrum from CNS modeling to repair (Figure 1). First, we provide a description of the native neural stem cell microenvironment in the developing and adult brain. Next, we review the range of advanced materials engineered to mimic the tissue architecture in vivo for improved in vitro models of the CNS for basic biology studies. Then, we shift to the translational use of materials for improved manufacturing and implantation of stem cell derived therapeutics for unmet needs in CNS diseases and disorders. Finally, we discuss regulatory considerations for advanced materials in stem cell therapeutics.

2. The Microenvironment of Stem Cells in the Central Nervous System

2.1. The Neural Stem Cell Niche in the Developing CNS

All mature neural cell types arise from neural stem cells in the developing embryo. During embryonic development, stem cells within the developing neural tube are exposed to a precisely orchestrated combination of extrinsic signals that compose the cell’s niche. Extrinsic signaling cues activate specific receptors on the cell surface that relay microenvironmental information to the cell nucleus by way of intracellular signal transduction cascades. Intrinsic determinants of cell fate, such as receptor expression profile, subcellular localization of organelles,[15] or epigenetic state of DNA,[16] modulate this cellular response to external signals. In the nucleus, gene transcription is initially regulated in response to the external environment on a short timescale (minutes-hours). Cumulative changes in transcription over long time scales (hours-days) then emerge as a discrete “output” phenotypic response, such as cell cycle arrest, cell division, differentiation into a specialized cell type, or cell death. Spatially overlapping gradients of different morphogens and growth factors[17] act to progressively pattern the diverse range of cell types in the brain and spinal cord.[18] The key morphogens that regulate spatial patterning of the neural tube include sonic hedgehog (SHH), WNTs, and bone morphogenetic proteins (BMPs)—which form antiparallel signaling gradients along the dorsal-ventral axis in the developing tube—

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and fibroblast growth factors (FGF), WNTs, and retinoic acid, which pattern the rostro-caudal axis.[18]

The extracellular matrix (ECM) of the stem cell niche also has critical functions in neuronal differentiation, maturation, migration, axonal growth, and synaptogenesis.[19,20] It is composed of a complex mixture of fibrous proteins, proteoglycans, and glycosaminoglycans (GAGs)—which include molecules such as laminin, collagen, gelatin, heparan sulfate, and fibronectin—many of which present peptide or glycan motifs that engage adhesion receptors on the cell surface and activate intracellular signaling pathways. During development, laminins are the earliest expressed matrix proteins, detected as soon as the two-cell stage of the developing embryo. The inner cell mass of the human embryo presents laminin 511 and 521, which are able to bind the integrin receptors expressed by embryonic stem cells (ESCs).[21] In the developing CNS, NSCs span the entire depth of the anterior region of the forebrain, and ECM signals likely arise from both apical and basal surfaces of the CNS. However, cell bodies are closer to the ventricular surface, in the VZ, rendering the signals within the VZ of particular interest. Many different laminin chains are expressed in this region, especially the alpha2 and 4 chains of laminin 111. In addition, high levels of chondroitin sulfate GAGs and chondroitin sulfates have been identified in these embryonic proliferative zones.[22] As the regions of the CNS continue to develop, the biochemical and biophysical signature of each region become distinct, and further instruct cellular fate commitment and maturation.

2.2. Structural Complexity of the Adult CNS

Rostro-caudal patterning of the neural tube during embryogenesis gives rise to the four regions of the CNS: forebrain, midbrain, hindbrain, and spinal cord. The forebrain is the largest brain division, composed by the four lobes of the cerebral cortex, and is responsible for a diverse range of functions including muscle movement, memory, thinking, decision-making, processing sensory information, receiving and processing visual information from the retina, auditory perception, memory formation, and language production as well as the regulation of the endocrine system. Cortical excitatory glutamatergic neurons, inhibitory striatal medium spiny neurons, GABA interneurons of the cortex, radial glia, oligodendrocytes, glutamatergic neurons, and astrocytes are among the cell subtypes responsible for the various functions within the forebrain.[23] The midbrain regulates motor movement, particularly movements of the eye, and aids in the processing of auditory and visual information. Several subtypes of dopaminergic neurons are present within the midbrain, and they closely interact with glutamatergic neurons, as well as glial cells.[24] The midbrain and hindbrain together compose the brainstem, which extends toward the spinal cord. The hindbrain assists in the regulation of autonomic functions, such as maintaining balance and equilibrium, movement coordination, and the relay of sensory information. The hindbrain is composed by baroreceptor- and glucose-sensitive neurons, glutamatergic neurons, as well as Purkinje cells and granule cells in the cerebellum.[25] The spinal cord, which functions primarily in the transmission of nerve signals from the motor cortex to the body, and from the afferent fibers of the sensory neurons to the sensory cortex, nucleates spinal interneurons, sensory neurons, motor neurons, and glial cells. Motor neurons are also found at different levels of the CNS, including the cortex, midbrain, and hindbrain.[26]

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Furthermore, the ECM surrounding cells within the adult brain is a complex macromolecular network composed of proteins and polysaccharides that occupy the space in between neurons and glia, and its composition changes throughout development and accounts for ≈20% of the total volume in the adult brain. These ECM molecules may be arranged diffusely to form an ECM matrix or condense to create specific compartments in the extracellular space, such as perineuronal and perisynaptic nets, or to form basement membranes in the neurovascular unit contributing to the blood–brain barrier. The main components of the ECM deposited in the extracellular space are hyaluronic acid (HA), sulfated proteoglycans, tenascin, and link proteins. In addition, the basic structure of the basement membrane is composed of laminin, enactins, collagen IV, and heparan sulfate proteoglycans (such as perlecan or agrin). The source of such molecules includes astrocytes as well as neurons, and astrocytes also secrete other ECM proteins, including SPARC, hevin, tenascin, and thrombospondin. These proteins are synthesized during development and have a reduced expression in the adult CNS. ECM glycoproteins secreted from neurons, such as reelin and leucine-rich glioma inactivated protein 1 (LGI1), act as signals both during development and in the adult brain that are critical for synaptic plasticity in the adult brain.[20] Overall, the complex structure of the CNS and intricate connections between different regions within constitute a big challenge for the development of reliable models that recapitulate its cellular diversity and ECM across various regions.

3. Materials to Enhance In Vitro Models of the CNS

3.1. Conventional Models of the CNS and Their Limitations

Several model systems have been employed to recapitulate human CNS development, physiology, and disease across a range of length scales from molecular to cellular to tissue level (Figure 2). 2D cultures offer systems that are simple, highly controllable, and amenable to high-throughput experimentation and data acquisition,[27] though they fail to reproduce the complex anatomy and physiology of natural tissues described in Section 2 because they lack control over the 3D spatial patterning of the cells as well as the spatio-temporal interactions between cells and their ECM.

On the other end of the spectrum, animal models of the CNS, such as rodents or non-human primates (NHP), provide the anatomy and physiology of a fully functioning CNS, including regional patterning and interactions with other organ systems such as the circulatory, endocrine, and immune systems. However, limited experimental throughput, genetic differences between rodents/NHP cells and human cells, and ethical considerations for use of animals limit the applicability of animal models.[28] Thus, there is a need for advanced in vitro models of the human CNS that are physiologically relevant, reproducible, controllable, and amenable to high-throughput applications.

3.2. Organoids for Enhanced Modeling of the CNS

3.2.1. What Is an Organoid?—Recently, 3D aggregates of hPSCs under specific environmental conditions have been found to execute developmental “programs” to create complex, organized multicellular structures that develop and mature in a manner that mimics fetal development. Termed “organoids,” these in vitro cellular aggregates can recapitulate

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histological features of various CNS regions,[29,30] as well as gene expression patterns and epigenomic signatures[31,32] and neural network activity.[33] Not only does their resemblance to human CNS development and disease[34] make organoids a more physiologically relevant model system compared to 2D cell cultures, but they also offer the capacity for both higher throughput studies and the ability to use human cells rather than animal models.[35]

3.2.2. How Are Brain Organoids Made?—Brain organoid protocols are divided into two major classes: i) self-patterning or unguided, whole brain differentiation that relies on intrinsic self-organization capabilities of PSCs[36] and ii) prepatterning or guided, region-specific differentiation, which drives cells toward a certain identity using small molecules and protein morphogens, which, for example, includes models of the forebrain[37] or midbrain.[38] In self-patterning protocols, cell aggregates, ideally of controlled size, are embedded in complex ECM scaffolds (e.g., Matrigel) that support neuroepithelium self-organization, and the resulting organoids exhibit characteristics of various cerebral regions. In contrast to self-patterning protocols, prepatterning methods use free-floating cultures of cell aggregates, and Matrigel is added to the culture medium. These organoids recapitulate many aspects of the early developing human neocortex.[39,40]

Currently, directed organoid differentiation protocols primarily rely on a specific sequence and duration of biochemical cues that are derived from specific stages of CNS development and are thus intended to generate the desired cell types/tissue by extrinsic regulation of stem cell fate (Figure 3A). For instance, when modeling neuroectoderm from hESCs, dual SMAD inhibitors (LDN193189 and SB431542), which block the activation of specific cell surface receptors, are used to both promote neuralization of the primitive ectoderm through BMP pathway inhibition and to suppress mesendodermal fate by inhibiting endogenous activin/TGFβ signaling pathways. From there, if, for example, generation of the forebrain region of the CNS is desired, a combination of WNT antagonist (such as DKK1) and SHH agonist (such as purmorphamine) is used to emulate the natural development of these cells that results from positioning within intersecting WNT and SHH signal gradients along rostro-caudal and dorso-ventral axes.[12]

3.2.3. Limitations of Current Organoid Protocols—Although the advent of organoid technologies has advanced in vitro models of the human CNS considerably, recapitulating certain cellular organizational structures of the developing CNS[36] and with proteomic similarities to fetal tissues,[41] there are still significant limitations in organoid reproducibility, biological maturation, and structural organization.

The early development of organoid technology relied heavily on Matrigel, a highly bioactive yet complex and poorly defined mixture of proteins and proteoglycans[42] extracted from mouse tumor cells (Table 1). Although Matrigel's composition offers an enriched environment for organoid growth and maturation, it has several drawbacks for future development of organoid models. Most prominent is the poorly defined composition of bioactive cues it contains, leading to difficulty in quality control measures and batch-to-batch variability, which may contribute to poor reproducibility and organoid consistency.[42,43] Additionally, the diverse biochemical and biophysical properties of a Matrigel scaffold are not well controlled, and it is difficult to parse the effects of any individual

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signaling cue from the many others present.[44] Physical properties that have been identified to influence stem cell fate, such as substrate stiffness, differ between Matrigel and the brain.[45]

Organoid formation studies in which cell aggregates were not embedded in supporting matrices, offered relevant insights into CNS biology, though organoid generation was not always reproducible.[46,47] These protocols, which utilize soluble cues in suspension cultures to provide biochemical instruction of stem cell fate decisions, limit spatial control of these signals and largely ignore important biophysical cues from the ECM that influence cell fate during CNS development.[40,48]

Biological limitations in current brain organoid protocols exist as well. Though important cell types of the CNS—including neurons, astrocytes, oligodendrocytes, and recently microglia—arise in current organoid models, the maturation of these cell types typically resembles that of fetal tissue rather than the adult CNS.[49,50] Additionally, the lack of symmetry breaking events during organoid maturation leads to inefficient lineage specification and disordered spatial arrangement of the different brain regions,[40,51] leaving unresolved questions about the maturation process of brain tissue and limited CNS disease modeling capability.

Structurally, the absence of vasculature in brain organoid systems constrains the size and therefore the development of additional neuronal layers because of low oxygen diffusion to the core, even creating a necrotic region in the center of many organoid constructs that may influence the physiology of adjacent cells. As a result, organoids have a limited capacity to replicate the complex multicellular organization and tissue-like architectures that arise during development within and across different CNS regions.

3.3. Advanced Materials to Guide Organization of Organoids

3.3.1. Toward Defined Material Scaffolds—Advanced material scaffolds can potentially be employed to overcome these limitations in current organoid protocols and specifically to help recapitulate the tightly tuned extrinsic environment of the developing CNS in vitro and thereby guide the patterning, growth, and maturation of organoids (Figure 3B) with progressively more defined and reproducible parameters (Table 2). Utilizing defined synthetic materials to culture organoids can provide the ability to isolate individual variables at a time to parse out its role in development. For example, a neural tube model in PEG investigated the role of retinoic acid, a biochemical patterning cue that plays a role in the developing CNS, and revealed that its role in inducing pattern formation was independent of any ECM or growth factor cues[52] (Figure 4A) when it was able to induce dorso-ventral organization in neuroepithelial cysts in a PEG matrix that was void of any ECM signals.

Hybrids of natural and synthetic polymers have also been used to incorporate essential signals from native ECMs, specifically tailored to create the cellular heterogeneity and maturation needed to recapitulate the desired CNS structures. The concentration, spacing, presentation, patterning, and timing of ligand presentation impact cell behavior and can be tailored using advanced material platforms.[40,48,53] For instance, a PEG-based hydrogel was

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used to systematically assess the role of a broad variety of potential extracellular modulators of neuroepithelial differentiation and morphogenesis, by cross-linking collagen IV, laminin 111, or perlecan, among others, into the PEG backbone.[54] Using organoids derived from mouse PSCs, the authors found that laminin was the most positive modulator of not only proliferation and neural differentiation of the progenitors but also of symmetry-breaking events that ultimately led to neural tube-like patterning along the dorso-ventral axis.

Furthermore, the conjugation of matrix metalloproteinases (MMP) cleavage sites in these PEG polymers allowed tuning not only of the chemical and physical (polymer rigidity) inputs to the developing organoid, but also a temporal change in polymer rigidity as the polymer was degraded over time.[54]

Defined materials have also been studied to physically guide the spatial patterning and growth of cerebral organoids. Specifically, poly(lactic-co-glycolic acid) (PLGA) microfilaments were used as a template for PSCs attachment, leading to polarization of the cell's physical environment in the presence of Matrigel together with defined soluble biochemical cues in the culture media.[55] The resulting elongated embryoid bodies (EB) enhanced neuroectoderm formation and subsequent cortical development (Figure 4B).

Additionally, increased reproducibility in CNS organoid generation has been achieved by using miniaturized multiwell spinning bioreactors that are able to grow larger organoids by improving diffusion of oxygen and nutrients, in a reduced culture volume system.[56] Using this method, the authors were able to generate forebrain, midbrain, and hypothalamic organoids from hiPSCs.

3.3.2. Novel Materials to Improve Vascularization for Enhanced Organoid Development—

Defined material scaffolds can potentially also be coupled with other advanced technologies to improve guided organoid development and thereby better control in vivo tissue functionality and complexity. For example, grafting organoids into living mice can introduce a vasculature system to better recapitulate an in vivo system,[57] though this approach essentially introduces previously described downsides of using a rodent model to study the CNS. As an alternative, a material based system that harnesses VEGF potency to pattern blood vessel formation and microfluidics to circulate oxygenated medium may be of future interest. Along this vein, two-photon patterning has been used to spatially arrange NGF in a 3D matrix that enabled researchers to guide the growth of neuronal axons during morphogenesis.[58] Alternatively, new synthetic-protein hybrid materials[59] or 3D printing[60] have potential to be employed for patterned immobilization of VEGF to guide angiogenesis within brain organoids, or other patterning cues to better recapitulate developmental morphogen gradients in the CNS.

3.3.3. Future Considerations for Material Scaffolds in Brain Organoid Protocols—

There are several existing challenges in brain organoid development that novel materials could be engineered to overcome. To mimic the combinatorial signaling environment of the developing CNS, morphogens such as retinoic acid and WNT could be immobilized into inert 3D scaffolds in perpendicular and antiparallel gradients. The scaffold material may also better mimic the biochemical and biophysical signaling environment of

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the neural stem cell niche by including defined combinations of ECM proteins as described previously in Section 2.2.[61] Given the dynamic microenvironment of cells in the developing CNS, these engineered niches would also benefit from an ability to modulate the signaling milieu over time, such as with microfluidic perfusion through a 3D scaffold to temporally control biochemical gradients and recapitulate the evolving niche at different stages of development as a stem cell turns into a progenitor and ultimately into a more mature and functional cell type.[62] Additionally, the introduction of automated and higher throughput culture systems will help realize the potential of brain organoid models in collecting well-controlled and robust datasets to better probe unresolved questions in CNS development and disease.[63]

Overall brain organoids offer a promising approach for generating physiologically relevant models of the human CNS amenable to high-throughput analysis. Combining current organoid protocols with advanced material technologies may improve the reproducibility, structural organization, and maturation issues evidenced by the use of exogenous biochemical cues alone. The resulting advanced organoid models of the CNS may enable applications in modeling development, neurodegenerative diseases, and in vitro screens for drug toxicity and potency.

4. Materials to Facilitate Cell Therapy for the CNS

4.1. The Promise of Cell Therapy for Unmet Needs in CNS Disease

In addition to using stem cells to increase our fundamental understanding of the CNS, they can also be harnessed to address unmet needs in human health. Stem cell-derived therapies are promising candidates to treat CNS disorders, and the rationale and feasibility of these therapies have been widely explored during recent years.[10,11,13,14] CRTs offer the potential to restore function in the CNS when neurodegenerative conditions have progressed to the stage that significant endogenous cell populations have been lost.[64] In addition, implanted cells could also secrete protective factors to slow disease progression.

Currently, there are several stem cell-derived therapies targeted to treat CNS disorders either in late preclinical development or clinical trials. Parkinson’s disease (PD) is estimated to affect 630 000 to 1 000 000 people in the United States (0.3% of the general population) [6,65,66] and involves the death of specific populations of neurons, including midbrain dopaminergic (mDA) neurons of the substantia nigra, which is accompanied by substantial motor and to an extent cognitive deficits in affected patients.[6,65,66] Therapeutic options for these patients are very limited and only provide temporary alleviation of motor symptoms. There are currently six ongoing clinical trials in the United States using mesenchymal stem cells (MSCs) to treat PD, presumably harnessing secretion of protective factors. Furthermore, methods to convert PSCs into mDA neurons have fortunately been developed, [67] and clinical trials using such PSCs-derived mDA neurons will also initiate soon.[68] As another important target, spinal cord injury (SCI) has a prevalence in the United States ranging from 240 000 to 337 000.[6] The pathophysiology associated with SCI involves a complex cascade of events including edema, hemorrhage, inflammation, severing of axons, parenchymal cavitation, and loss of myelin-producing oligodendrocytes.[69] Given the severe functional deficits and the current lack of treatment options to aid neurological recovery,

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cervical SCI remains an important research focus for regenerative medicine, including stem cell based replacement therapies to remyelinate the damaged spinal cord.[69] There are currently five ongoing clinical trials in the United States involving the use of stem-cell derived products such as PSCs, NSCs, and MSCs to treat SCI. Furthermore, a clinical study involving the use of hESC-derived oligodendrocyte precursor cells (OPCs) just completed Phase 1/2.[68] These efforts promise to substantially advance the cell therapy field.

While patient-derived autologous cells would be the immunologically ideal cell therapy,[70] it is very challenging to isolate sufficient numbers of such cells for an effective treatment,[71] and the majority of human somatic cells can undergo only limited expansion.[64,72] Therefore, this level of personalized medicine can be logistically and economically challenging. Allogeneic hESC and hiPSC-based therapies offer a potential solution to this challenge—particularly for generating sufficient numbers of cells for conditions with large patient numbers such as PD, SCI, or age-related macular degeneration (AMD).

4.2. Roadblocks to Effective Translation of Stem Cell Therapeutics

4.2.1. Current Challenges in Stem Cell Manufacturing—To effectively harness the full potential of stem cell-derived therapeutics, robust, reproducible, and scalable manufacturing practices are needed to produce high-quality, potent cellular products that meet critical quality attributes (CQAs), such as identity, purity, and potency of the cell product.[73,74] Stem cell manufacturing for CRTs requires a very tightly controlled microenvironment where soluble factors, topological cues, and spatial organization give rise to specific cell population of interest, initially through cell expansion and then lineage-specific differentiation.[75] For example, mDA neurons derived from hiPSCs using the FoxA2 floor plate method are advancing to clinical trials for PD. This method involves seeding stem cells in a 2D environment on a Matrigel-coated surface, then exposing them to dual SMAD inhibitors, SHH, and WNT agonists for 10 d to induce FOXA2+/LMX1A+ midbrain floor plate precursors for mDA neuron differentiation.[67,76] As another example, for clinical development of OPCs for SCI, hESCs are seeded into ultralow attachment flasks to stimulate EB formation. After a month in culture, the EBs are plated in a 2D environment on Matrigel-coated flasks, and additional differentiation factors are added to trigger OPC differentiation.[69] As the stem cell therapy field naturally and progressively matures, future manufacturing practices may benefit from addressing several challenges in the clinical application of stem cell-derived products.

One challenge is the limited reproducibility associated with batch-to-batch variability of tissue-derived products like Matrigel. Fully defined, synthetic materials offer the possibility of gaining better control and therefore higher reproducibility over manufacturing conditions. Another major challenge is scalability. Naturally derived compounds can be difficult to produce in large enough quantities, due to complex derivations (e.g., Matrigel from mouse tumor), limited availability (e.g., laminin from human placenta), and high costs. Moreover, natural compounds can be highly biodegradable and therefore need to be replenished during the production run.

Additionally, culturing cells in 2D is labor-, space-, and reagent-intensive and is thus generally best suited for generating small cell numbers for early stage clinical development.

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[77–79] As an example, typically ≈2 × 10^5 cells can be produced per cm^2 of 2D surfaces, such that it would require ≈1 × 10^7 cm^2 (equivalent to ≈180 000 10 cm cell culture dishes) to hold just the ≈2 × 10^{12} mDA neurons to treat nearly all patients with PD in the United States (with a post-implantation survival rate of ≈1–5%, ≈2 × 10^6 cells are needed per patient to guarantee the survival of 105 cells needed for the therapeutic effect),[80] not accounting for substantial additional surface area for cell expansion, the <100% efficiency of cell differentiation, batch testing and patient administration.[81,82] For SCI, ≈2 × 10^7 OPCs per patient are needed, requiring ≈6 × 10^5 10 cm cell culture dishes (≈4 × 10^7 cm^2) to manufacture just the OPCs used to treat the prevalent U.S. population.[69] Together with this cost-inefficient process to scale out 2D cultures, large-scale manufacturing of stem cells in 2D also presents numerous additional challenges such as modest expansion rates of stem cells (≈4–10×/passage) as well as limited control over cell spontaneous differentiation triggered by agglomeration.[75] Additional remaining challenges related to 2D cultures include poor viability upon cell dissociation and passaging (which involves mechanically dissociating highly delicate cells that are in tight contact with the surface).[83,84] Furthermore, 2D cultures are very different from the physiological microenvironments of many cell types. The lack of the natural tissue architecture, which often times translates into non-natural cell polarity and cell–cell contacts, can lead to suboptimal survival, expansion, and differentiation.

3D suspension cultures, such as with low-attachment plates or stir-tank bioreactors where cells are free-floating in the culture medium, have been extensively studied as a means to scale up cell production.[77–79,83] However, a critical problem with these cultures is uncontrolled cell agglomeration. hPSCs are prone to form strong cell–cell interactions that lead to aggregation within this type of 3D environments.[85] Agglomeration leads to inhomogeneity in cell aggregate, making it a poorly reproducible and inefficient process.[78] The transport of oxygen, nutrients, and growth factors to—as well as the transport of metabolic waste away from—the interior of large agglomerates (>400 μm diameter) becomes limited, leading to slow growth, cell death, and uncontrolled differentiation.[78,86] While increasing agitation in stir-tank bioreactors can reduce cell agglomeration, it also exerts hydrodynamic, shear stresses that compromise cell survival.[78,87,88] Therefore, these suspension-based 3D cultures can lead to low reproducibility and inefficient scalability (low volumetric yields).[83]

4.2.2. Current Challenges in Implantation of Stem Cell-Derived Products—

The promise of CRTs for the treatment of degenerative disorders within the CNS is contingent upon the survival and functionality of the replacement cell population after delivery to the injury/disease site in the CNS, yet several technical and biological hurdles peri- and post-implantation pose challenges for successful delivery of CRTs.

The primary mode of delivery of cell therapies to the CNS is by intracranial or intrathecal injection in order to localize the transplant to the site of degeneration or injury, yet several technical hurdles challenge efficient cell delivery. Implantation of mature hPSC-derived mDA neurons typically results in extremely low cell survival postimplantation (1–5%).[81,82,70,89] During the injection process, cell suspensions within a syringe are prone to sedimentation during the waiting period between loading cells into the syringe and the

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injection,[90,91] and the pressure created during the injection process has resulted in reflux, or backflow, of cells along the injection tract.[92,93] Both sedimentation and reflux during injection can result in cell dosing inconsistencies. Furthermore, cells experience an abrupt velocity increase from the syringe to the needle, as well as high shear forces from velocity gradients of flow through the relatively narrow needle, that may affect cell viability, especially for more mature cell types with neurites that may be more sensitive to shear.[94] Unfortunately, implantation of less mature, more robust neural progenitor cells may result in off-target differentiation or even continued cell division in vivo, which pose safety risks. These injection-related hurdles may be bypassed for specific cases of injury, such as traumatic brain injury (TBI) or SCI, if a surgical engraftment is possible.

Postimplantation, cell grafts face additional threats to viability, maturation, and functionality from several sources in the host tissue.[66] Treatment benefits for PD patients from CRTs are typically only noticeable at ten months to two years or longer after transplantation.[81,82] This slow alleviation of disease symptoms is mirrored in preclinical models, where animals show significant behavioral or motor improvements only several months post-transplantation,[93,95–98] and one potential cause is that transplanted progenitor cells likely need to mature further into functional mDA neurons in vivo before releasing dopamine and innervating surrounding neurons. Depending on the magnitude of disease progression or injury in the host tissue, the microenvironment may be void of trophic support that encourages cell engraftment and function, or even contain inflammatory or toxic factors secreted from reactive astrocytes[98–100] or microglia that contribute to graft rejection by the host immune system in the CNS, especially for allogeneic cell grafts.[101–104] Subsequently, over longer timescales after implantation, cells that do not innervate or migrate outward from the injection site or seek out vasculature may function suboptimally, if at all, due to insufficient oxygen/nutrient exposure and thereby reduce the potency of the overall cell graft.[105]

4.3. Novel Materials to Overcome Current Challenges in Stem Cell Manufacturing and Implantation

In the last decade, there has been a steady increase in the number of engineered materials designed to optimize stem cell production in a variety of cell contexts and for diverse applications in the CNS. Tables 3 and 4 summarize the CQAs for each desired cell type with the potential to target different CNS conditions and the novel materials designed to overcome some of these challenges. In this sense, advanced materials bring several advantages for stem cell manufacturing since they offer the possibility to mimic biochemical, mechanical, and topological features of natural environments in 2D or 3D (Figure 5). Along this vein, implantation of cells with materials shows potential in alleviating one or more of the current problems in cell transplantation in the CNS. Generally, the material acts as a multifunctional delivery vehicle for the cellular cargo during implantation (Figure 6). However, it is unlikely a one-size-fits-all material can be constructed for the range of CRTs being developed for CNS repair. For example, retinal pigment epithelium (RPE) cells for treatment of AMD showed enhanced anatomic integration and functional activity from implantation of a monolayer on a synthetic parylene substrate that mimics the structure of Bruch’s membrane, compared to RPEs in suspension.

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[106–108] Conversely, mDA neuron grafts in PD rodent models displayed enhanced cell survival and innervation if transplanted within a 3D hydrogel scaffold containing trophic cues.[109] Advantageously, materials can be engineered to exhibit a diverse range of chemical and physical properties that can be customized for a specific purpose, CQA and implantation region in the CNS, as we describe next.

4.3.1. Natural versus Synthetic Materials—

Materials for stem cell manufacturing and implantation are typically made of polymers that can be classified into two main categories, natural and synthetic.[110] The most commonly used natural or naturally derived materials are made of collagen, alginate, hyaluronic acid, chitosan, methylcellulose, or decellularized tissues.[110] These materials offer biocompatibility and bioactive properties that make them attractive for several tissue engineering applications.[111] However, low stability, limited control over mechanical properties, and at times rapid biodegradation are disadvantages of such naturally derived materials.[112]

Synthetic materials are chemically defined polymers that can offer several advantages such as: a) tunability of mechanical properties over a wide stiffness range, b) greater control over material degradation, c) low batch-to-batch variability and thus high reproducibility, d) cost-effective scalability, and e) biological inertness, which can be advantageous for their application in stem cell manufacturing when a low interaction with biological molecules is desired. However, lack of cell adhesion sites and potential toxicity can be disadvantages of these synthetic materials[113] that necessitate materials engineering and optimization efforts. For example, materials can be tailored to exhibit different physical properties, such as topography or stiffness, or diverse biological traits, such as biodegradability or the incorporation of bioactive cues to increase survival, proliferation, differentiation, or maturation of stem cells.[114] Some examples of synthetic materials are poly(ethylene glycol) or PEG, poly(N-isopropylacrylamide) or PNIPAAm, poly(ε-caprolactone), PLGA, poly-lactic acid (PLA), poly-ε-caprolactone (PCL), and polystyrene (PS). Natural and synthetic materials can be combined in a variety of ways to form hybrid materials that harness the strengths of each constituent.[115]

4.3.2. Materials for 3D Cell Encapsulation

Thermoreversible Cell Encapsulation: For cell manufacturing, synthetic, chemically defined, and thermoreversible PNIPAAm-PEG materials have been shown to support hPSCs survival, as well as expansion in growth media followed by differentiation upon addition of instructive cues, in a 3D environment that minimizes agglomeration due to physical separation of cells within the gel (Figure 7A,B).[80,83,116] This hydrogel presents the advantage of having a sol-gel transition temperature of ≈25 °C, allowing for mixing cells with the material as a liquid while on ice followed by cell encapsulation upon elevation to 37 °C. hPSCs grown in these conditions can achieve densities of up to $\approx 2 \times 10^7$ cells mL^{-1} of hydrogel, whereas the most effective 2D system enables a final yield of $<1 \times 10^6$ cells mL^{-1}. [83] This hydrogel has also been used to culture hPSCs in bioreactors by simple extrusion through a syringe to form fibers. This method increased cell survival compared to standard 3D bioreactors with free-floating cells by both reducing hydrodynamic stresses and ensuring efficient nutrient transport via controlling the cluster size of expanding cells to less than 400

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μm (radial diameter).[80] Together with higher cell yields and scalability compared to other culture platforms, this system also adds more control over stem cell differentiation by both alleviating agglomeration as well as allowing gentle cell retrieval by simply adding cold media to the hydrogel followed by partial dissociation of the cell clusters, removing the need for enzymatic cell harvesting and minimizing chemical and physical stresses.

For cell implantation, thermosensitive hydrogels can exhibit a sol-gel transition in between room temperature and body temperature. This temperature responsiveness enables feasible handling to load into a syringe while in the liquid phase, and cell encapsulation in the gel phase when heated and ultimately implanted at body temperature. Extensive phase equilibria studies and models for polymer-solvent systems have been established and may help inform design and predict phase behavior for use in injectable stem cell therapeutics.[117] In practice, NSCs suspended in a thermosensitive diblock copolypeptide hydrogel (DCH-T) above the lower critical solution temperature (LCST) exhibited less sedimentation and clumping in a syringe compared to aqueous solution, as well as increased survival after injection[118] (Figure 8A). The increased viscosity of the injection as it formed a gel at body temperature in situ can also mitigate backflow of cells up the injection tract by imposing a physical containment (Table 5).

Chemically Cross-Linked Materials for Cell Encapsulation: Natural or synthetic polymers that form hydrogels using chemical cross-linkers can also be used for cell encapsulation during manufacture or implantation. Alginate is a natural anionic polymer electrolyte polysaccharide extracted from brown algae.[110] It is a strictly linear copolymer composed of two monosaccharides of α-L-guluronic acid (G) and β-D-mannuronic acid (M), respectively.[119] Alginate forms a dense 3D hydrogel when exposed to divalent ions, such as Ca^{2+}, Sr^{2+}, and Ba^{2+}, and could therefore be employed to encapsulate cells using standard culture media conditions. hESCs grown in alginate gels have been shown to proliferate and expand over several passages while retaining their pluripotency (Figure 7C–E). To recover cells from this hydrogel, medium is removed, and alginate hydrogels are dissolved with EDTA.

Furthermore, alginate possesses an advantageous shear-thinning property where the viscosity of the gel decreases under strain, such as when pushed through a syringe during cell implantation, but increases back to its original viscosity when the strain is removed. In particular, the regions of the gel closest to the inner wall of the needle undergo shear thinning to lubricate the motion of the rest of the bulk hydrogel as it passes through the needle in a plug flow manner. The cells within the bulk of the gel distal from the inner walls of the needle are in theory insulated from high shear and deformation during the velocity increase from syringe to needle that may otherwise lead to cell death. For example, MSCs and neural progenitor cells encapsulated in alginate showed improved viability when passed through a needle in comparison to cells in PBS.[120] HA exhibits similar shear thinning as observed when fibroblasts encapsulated in a HA and methylcellulose blend were injected into a model of SCI resulting in increased viability post-transplantation.[121]

4.3.3. Material Stiffness and Topography—

The physical microenvironment has a large influence on yield of the target neural cell type during stem cell manufacturing.

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Mechanistically, physical inputs are relayed through transmembrane mechanotransduction receptors such as integrins and mucins and downstream targets, such as Rho GTPase and Yes-associated protein (YAP) signaling pathways.[122,123] The stiffness of the normal adult brain varies among its different regions, with an average range between 1 and 2 kPa,[124,125] although certain regions such as the neurogenic niche in the hippocampus have been shown to fall between 50 and 120 Pa.[126] To recapitulate the physical microenvironment of endogenous neural stem cells, material stiffness can be tuned by modulating the composition, concentration, and degree of crosslinking of base polymers, and can range from very soft (<0.1 kPa) to very stiff (>100 kPa). Substrate stiffness has been shown to control neural stem cell proliferation and differentiation programs. Soft matrices promote NSC differentiation into neurons, whereas stiffer substrates promote astrogenesis.[127,128] These results are analogous to studies on mechanosensitive differentiation of MSCs, which showed that soft substrates favor adipogenesis whereas stiff substrates promote osteogenesis.[129]

Studies of mechanosensitive neural stem cell differentiation have been conducted in both 2D, when seeded on polyacrylamide-based hydrogels[127] and chitosan-based hydrogels,[130] as well as 3D,[131,132] when encapsulated in scaffolds such as alginate hydrogels.[133] For example, alginate hydrogels with a stiffness range between 180 and 20 000 Pa were used to encapsulate NSCs, and neuronal differentiation was promoted in soft hydrogels (≈180 Pa).[133] This finding was consistent with reports using 2D platforms in which neuronal differentiation was favored in the softest gels (≈500 Pa).[134] While both of these 2D and 3D studies identified the pro-neurogenic stiffness to be close to the stiffness range found in the stem cell niche within the hippocampus, the brain region where these cells reside, the 3D model more closely recapitulated the mechanical properties as well as cytoskeletal arrangements of these cells in their natural 3D environment.

Topographical features, such as porosity, also affect stem cell proliferation, migration, and differentiation.[134,135] Several engineered materials can be tailored to exhibit controlled topographical properties for stem cell manufacturing. Teng et al. used sphere-templating fabrication techniques to make controllable porous hydrogel scaffolds and measured the toughness (ability to plastically deform without fracturing) of synthetic hydrogels compared to their porous counterparts. The toughness ranges of many synthetic hydrogels are in the range of 10^4–10^5 J m^{-3}, whereas the modified porous hydrogel scaffolds presented a toughness of 1.5×10^6 to 1.4×10^7 J m^{-3}, showing how material porosity affects the plasticity properties of these materials.[135] Using porous hydrogels, another study showed that high porosity hydrogels increase proliferation of NSCs and promote glial cell over neuronal differentiation.[136] In addition, porous PEG hydrogels allow for spindle-shaped cell morphologies comparable to natural fibrin, supporting the notion that porosity and overall substrate morphology can affect stem cell shape, adhesion, migration, and ultimately fate. It has also been shown that adhesion of hMSCs to hydrogels with heterogeneous surface wrinkles changes their shape and differentiation patterns.[137] Future studies may extend these findings to cell manufacturing from hPSCs.

The physical and topographical properties of polymer hydrogels can also be tuned to aid cell transplantation in the CNS by modulating variables such as polymerization time and density

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of cross-linking sites in the base polymers (Table 6). Pore sizes for implantation materials have ranged from hundreds of nanometers[138] to hundreds of micrometers in diameter.[139] Smaller pores can function as a physical barrier to host microglia and macrophage invasion during an inflammatory response to transplantation or in conditions such as TBI where the reactive environment is a secondary cause of cell death after the primary injury (Figure 8B).[138] Conversely, higher porosity has been implicated in blood vessel formation,[140] which is a key consideration for cell grafts and tissue regeneration. Pore size can increase when polymer scaffolds are broken down by either nonenzymatic or enzymatic processes.[141] Notably, hydrogel porosity is a property that can be engineered to vary over time by tuning the degradation kinetics of the polymer.

4.3.4. Engineering Materials with Tunable Biodegradability—While PNIPAAm-PEG and alginate materials form nonconvalently crosslinked gels, other materials involve covalent crosslinking. As a result, biodegradability must be considered to evaluate their potential use in stem cell manufacturing. On one hand, degradation provides the space for cell proliferation and tunable, progressive release of encapsulated trophic factors.[142] Furthermore, degradable materials reduce the deleterious effects of physical confinement on cell differentiation.[143,144] However, subproducts of degradation may be toxic or bioactive, which needs to be carefully controlled and taken into account upon the initial material design. In addition, controlling a material's degradation rate can offer better reproducibility.

Naturally derived materials are typically biodegradable and nontoxic. However, their degradation kinetics in culture can be hard to control, especially when the cells secrete degradative enzymes, which often makes them less ideal for cell manufacturing. Such is the case of alginate, HA, or gelatin. Several approaches have been developed to gain control over degradation of these materials. Ashton et al. demonstrated that embedding a controlled release alginase inside of an alginate hydrogel for tunable enzymatic degradation could significantly stimulate encapsulated NSC proliferation compared to standard alginate.[145] Analogously, gelatin can be degraded by many secreted metalloproteases in culture,[146] and hybrid hydrogels conjugating gelatin to synthetic polymers have been developed to gain control over its degradability.[147]

Synthetic hydrogels are often non-biodegradable but can be modified by crosslinking them with degradable molecules to enable controllable biodegradation. For example, several groups have made NIPAAm copolymers biodegradable by synthetizing a type of thermoresponsive NIPAAm copolymer with hydrolysable lactate ester side groups.[148] As another example with PVA, the incorporation of hydrolytically labile ester can trigger the degradation of polymer networks at neutral pH.[149] On the other hand, PEG-based hydrogels have been widely used for different applications to exert diverse biodegradability properties. Examples of this are photodegradable hydrogels[150] or PEG-based hydrogels that degrade in response to secreted MMPs enabling tunable hMSC culture.[151] Furthermore, a photodegradable and biocompatible hybrid hydrogel made of gelatin and PEG offers the possibility of spatially and temporally tuning its physical and chemical properties with light exposure, without compromising the integrity of the cells.[152]

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Biodegradability is a critical aspect of polymers used to aid cell implantation into the CNS. Nonenzymatic degradation occurs for a class of pH-sensitive synthetic polymers, such as hydroxyethyl-methacrylate (HEMA), that degrade into nontoxic biocompatible components at endogenous pH levels. The synthetic nature of these polymers offers the advantages of a fully defined chemical matrix. Alternatively, enzymatic cleavage sites can be chemically incorporated into polymer backbones, such as the β1–4 glycosidic bonds of HA units in GAG polymers that are cleaved by hyaluronidases present endogenously in the brain, or peptide sequence recognized by zinc-dependent MMPs secreted from cells. A HA-based scaffold with MMP cleavage sites promoted formation of vasculature in the NSC graft site of a stroke model (Figure 8C).[141] In addition, MMP kinetics can be quantified to predict material degradation time and infer subsequent cell response in order to guide polymer design of materials implanted with cells.[153]

4.3.5. Engineering Materials with Customized Bioactivity—Base polymers can be engineered to possess specific bioactive moieties to further instruct a desired cell response. Covalent tethering of cell responsive peptides or growth factors onto base polymers influences both the stability of the cue and its presentation to the cells, which can enhance cell production. For cells that are dependent on some form of ECM anchor for survival, cell-adhesive peptides such as RGD or IKVAV can be conjugated to base polymers to prevent anoikis. Some examples of such materials—which also offer the advantage of tunable-stiffness—used for stem cell manufacturing are HAPNIPAAm-PEG,[116] PEG-norbornene-CRGDS,[154] or alginatechitin.[155] Among materials engineered to present additional bioactive cues, some examples are decellularized plant tissue scaffolds coated with the fibronectin-derived integrin-binding domain, RGD,[156] or PS coated with polydopamine and PLGARGD together with trophic factors.[157] For cell implantation, this approach can provide supplementary functionality to enhance graft survival/integration, promote cell migration, or provide trophic support for cell maturation. NSCs transplanted in RADA16 with IKVAV sites showed higher survival and integration into the host brain tissue in a model of cerebral neocortex loss.[114]

Soluble bioactive cues, such as growth factors, can be added to base materials via electrostatic charges from polar functional groups, such as carboxylic acids, amides, sulfates, and hydroxyls that are abundant in naturally occurring biopolymers composed of repeating disaccharide units of GAGs such as HA, heparin, chondroitin sulfate; synthetic chemical polymers such as HEMA; and polyelectrolytes such as PCL, poly-L-lysine (PLL), and PLA. Growth factors with oppositely charged moieties exhibit sustained release due to decreased diffusion rates when enmeshed within these hydrogels. For example, transplantation of mDA neurons into rodent models of PD using a HA hydrogel mixed with trophic cues HGF, GDNF, and Ephrin-B2 enhanced graft survival, dispersion, and integration into the native neuronal network[109] (Figure 8D). However, immobilized growth factors may be a more potent and stable form of signal presentation than in soluble form[158] for several reasons. First, diffusion away from the graft site is prevented. Second, receptor-mediated endocytosis, whereby a cell internalizes a receptor-ligand complex resulting in degradation of the ligand, is hindered. In a study that compared PCL functionalized with immobilized GDNF to soluble GDNF, the immobilized GDNF remained bound and retained

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higher bioactivity than the soluble form in vitro, and also enhanced survival, proliferation, migration, neurite outgrowth of encapsulated NSCs in the brain parenchyma, as well as reduced inflammatory responses.[159]

Finally, new combinations of natural and synthetic materials with novel features for cell transplantation in the CNS have been developed and assessed for their functionality in vitro that may have potential for in vivo studies of safety and functionality. For example, a fully synthetic PEG-based hydrogel with fibronectin fragments that provide cell adhesive domains was found to guide linear neurite extension and is a candidate for NSC transplantation in SCI.[160] Additionally, naturally derived silk fibroin functionalized with laminin displayed enhanced and unidirectional neurite outgrowth.[161] Carbon nanotubes have been considered for guiding neuronal axon growth as well and demonstrate biocompatibility.[162] For NSC transplantation, a PEG-4MAL based polymer that used affinity based binding of laminin to N-terminal agrin (NtA) displayed higher bioactivity compared to alternative strategies to incorporate laminin into the synthetic polymer.[163]

4.4. Future Considerations for Material Scaffolds for Stem Cell Manufacturing and Implantation

There are several remaining challenges in stem cell manufacturing and delivery that can be addressed by engineering advanced materials. For example, limited control over cell differentiation during production leads to highly heterogeneous cell products with low yields of the desired cell types and poor reproducibility of the production process. Materials have the potential to overcome these limitations by incorporating spatiotemporal control over the biochemical and biophysical signals presented to these cells to more closely resemble their natural environment, as well as tunable degradability to offer cells enough space to grow while progressively releasing encapsulated trophic factors.[152,164,165] As a result, expansion of PSCs can be maximized with a reduced risk of spontaneous differentiation, and subsequent efficient specification into the desired cell lineages can be achieved. In addition, these materials need to be suitable for scalability, such as thermoreversible 3D scaffolds, in order to produce sufficient quantities of cells to treat affected patient populations. Another challenge associated with stem cell manufacturing is the need for reliable and cost-effective high-throughput analytics to determine identity, purity, and potency of the cell products in vitro, amenable to high-scale production.[166–168] Detailed characterization of lineage commitment dynamics of differentiating hESCs that has reached unprecedented resolution with the use of single-cell RNA-seq[169–172] will aid in the development of improved analytics as well as enhanced purification methods to obtain high yields of highly pure cell populations.[169–172]

Remaining challenges associated with implantation of stem cell-derived products into the CNS can also be tackled by engineering advanced materials. Controlling physical properties of delivery materials, such as viscosity or thermoreversibility, may reduce cell death and aggregation during delivery into the target site, increasing the efficacy of the therapy and reducing the variability between injections.[120,173] Upon transplantation, there is a need for tighter control over cell survival, differentiation, maturation, migration, and establishment of functional connections with the neighboring cells, which would benefit from the use of

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material scaffolds designed to incorporate biochemical and biophysical cues as well as controlled release of trophic factors in the engraftment site.[174,175]

Stem cell-derived products to treat neurodegenerative disorders have shown promising results in early stage clinical trials. Advanced materials for manufacturing and delivery of these cells offer the opportunity to improve quality, reproducibility, scalability, and safety of these products to increase their potential for clinical translation in order to meet patient needs and expand CRTs to broader applications in CNS diseases.

5. Regulatory Considerations for Clinical Translation of Materials in CRT Manufacturing and Implantation

Several moving parts need to converge to advance a cell therapy product to the clinic, and the complexity of the effort doubles if a unique delivery vehicle is needed. Following a risk-based approach in the early design and development of the cell and material components in the overall therapeutic is recommended whereby each subcomponent of the development pipeline is analyzed through a lens of mitigating risks to overall create a predictably manufacturable, safe, and efficacious therapeutic.

The wide array of choices available for polymer backbones and additives confers a vast design space in engineering material scaffolds with desirable properties to enhance cell manufacturing and transplantation to the CNS (Figure 9). Choice of starting materials can be a critical decision that needs early consideration in the development of cell and material therapeutics. For cells, the source of donor can greatly influence the production process. For autologous therapies especially, donor cells may exhibit wide heterogeneity and the results of a generic production process may vary widely and in an unpredictable manner. Quality control tests to understand the composition of a donor cell population, such as immunocytochemistry or gene expression analysis for defining cell markers are necessary to identify and mitigate risks from the onset. Recently, omics-based approaches for cell population classification have been employed for higher resolution information, though deriving meaningful interpretations will represent a challenge. For raw materials that will go into developing a delivery scaffold for cell transplantation, it is advantageous to use materials that have established quality control procedures, are deemed safe or even already FDA approved for biomedical purposes, can be mass manufactured, and are available from multiple vendors, rather than synthesizing the majority of starting materials in-house which may have wide variability from batch-to-batch of production, and will affect the synthesis downstream.

Scalability of the cell production and material synthesis processes should be considered early in the product development, perhaps in conjunction with early safety and efficacy tests, as manufacturing at clinical scale is the bottleneck for many cell therapies in clinical pipelines currently. Given the long production time of some cell therapies, within-process sampling of cells and media during differentiation processes are advantageous to measure and monitor to ensure the production is on track, or halt a run at the earliest sign of deviation from the expected operating range rather than waiting until the end of a full production run. At the macroscale, engineered materials for cell manufacturing will possess bulk properties

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that need to be considered when choosing a bioreactor configuration. For example, hydrogels that are delicate are not well suited for a wave or stirred tank reactor that will impose turbulent flow and eddies but may fit well with a plug flow reactor that imposes a slow perfusion of fresh media. Additionally, materials with controllable degradation, such as enzymatically cleaved or thermoresponsive materials, are advantageous for cell retrieval on demand for within-process monitoring as well as cell harvesting at the end of a production run.

Finally, sterilization processes for material delivery vehicles, such as gamma or UV irradiation, should be designed to not alter functionality of the potent components of the material.[176] UV-sensitive bonds in polymer networks may be a concern and alternative sterilization methods may need to be sought out, such as ethylene oxide or supercooled CO_2 treatment.[177]

6. Conclusion

Materials with a diverse range of properties have been engineered to interface with stem cells in a controlled and tunable manner to overcome limitations in current cell culture methods. The application of novel materials in basic biology helps probe fundamental questions about human CNS development by construction of advanced organoid models in vitro. Furthermore, novel materials can be applied to solve problems in manufacturing and implantation of stem cell therapeutics to effectively translate candidate therapies from the bench to the clinic.

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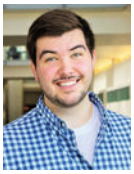

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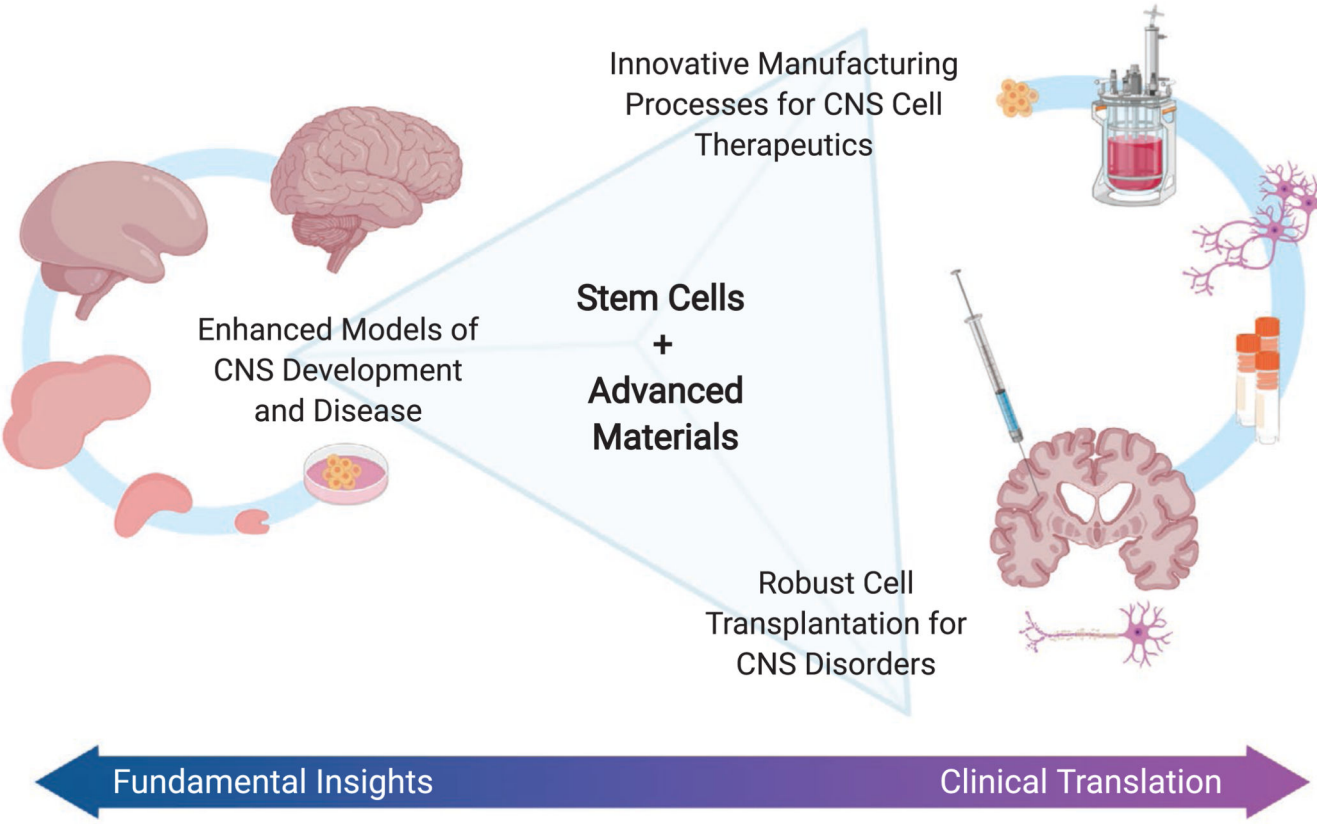

Figure 1.
Applications of advanced materials in CNS study and repair.

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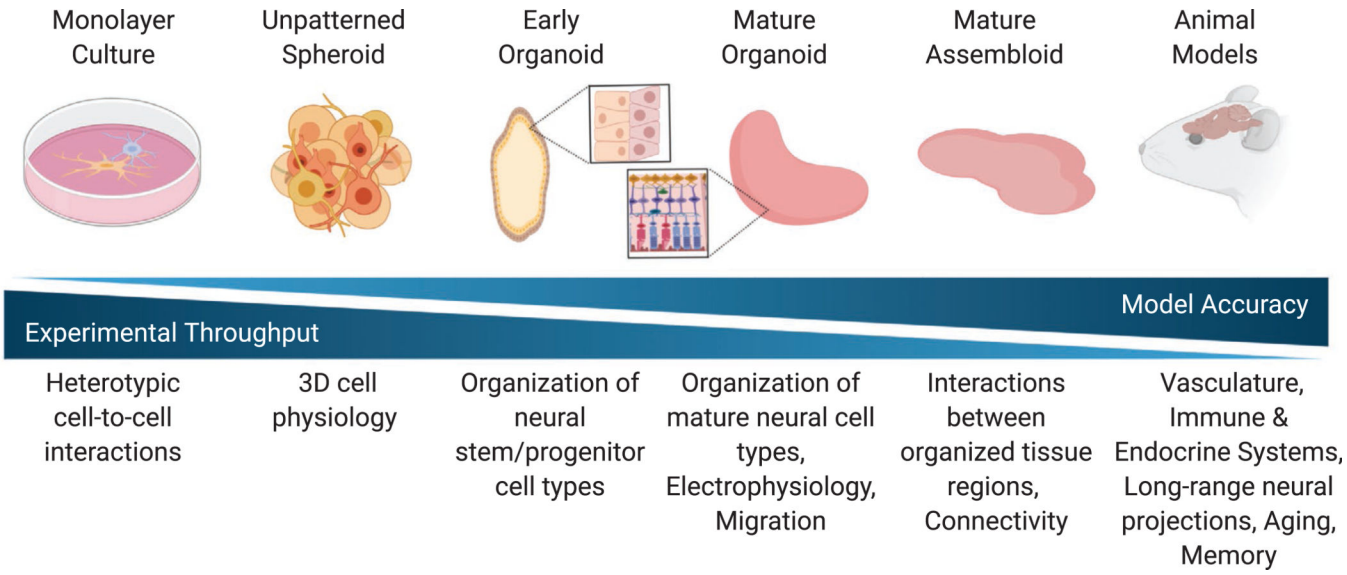

Figure 2.
Comparison of features for different models of human CNS development.

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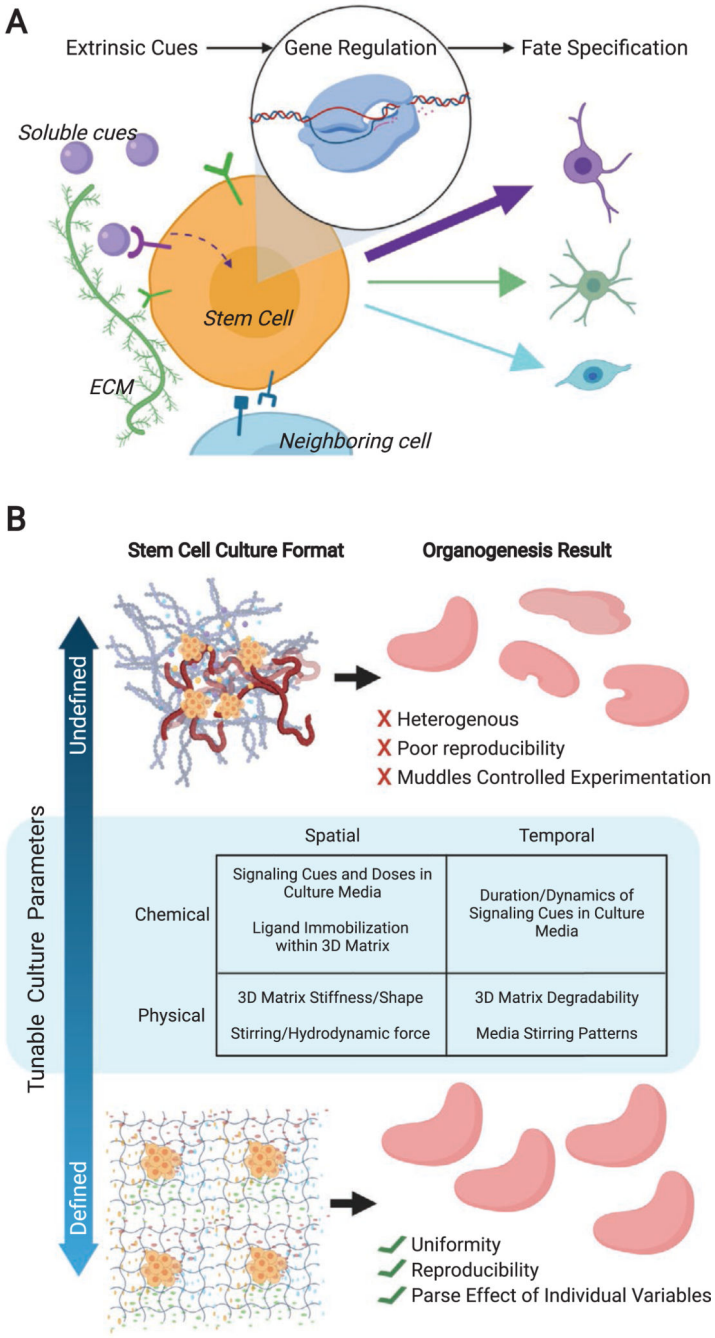

Figure 3.

A) Extrinsic regulation of stem cell fate. B) The use of material scaffolds to guide chemical and physical extrinsic regulation across space and time for improved generation of organoids.

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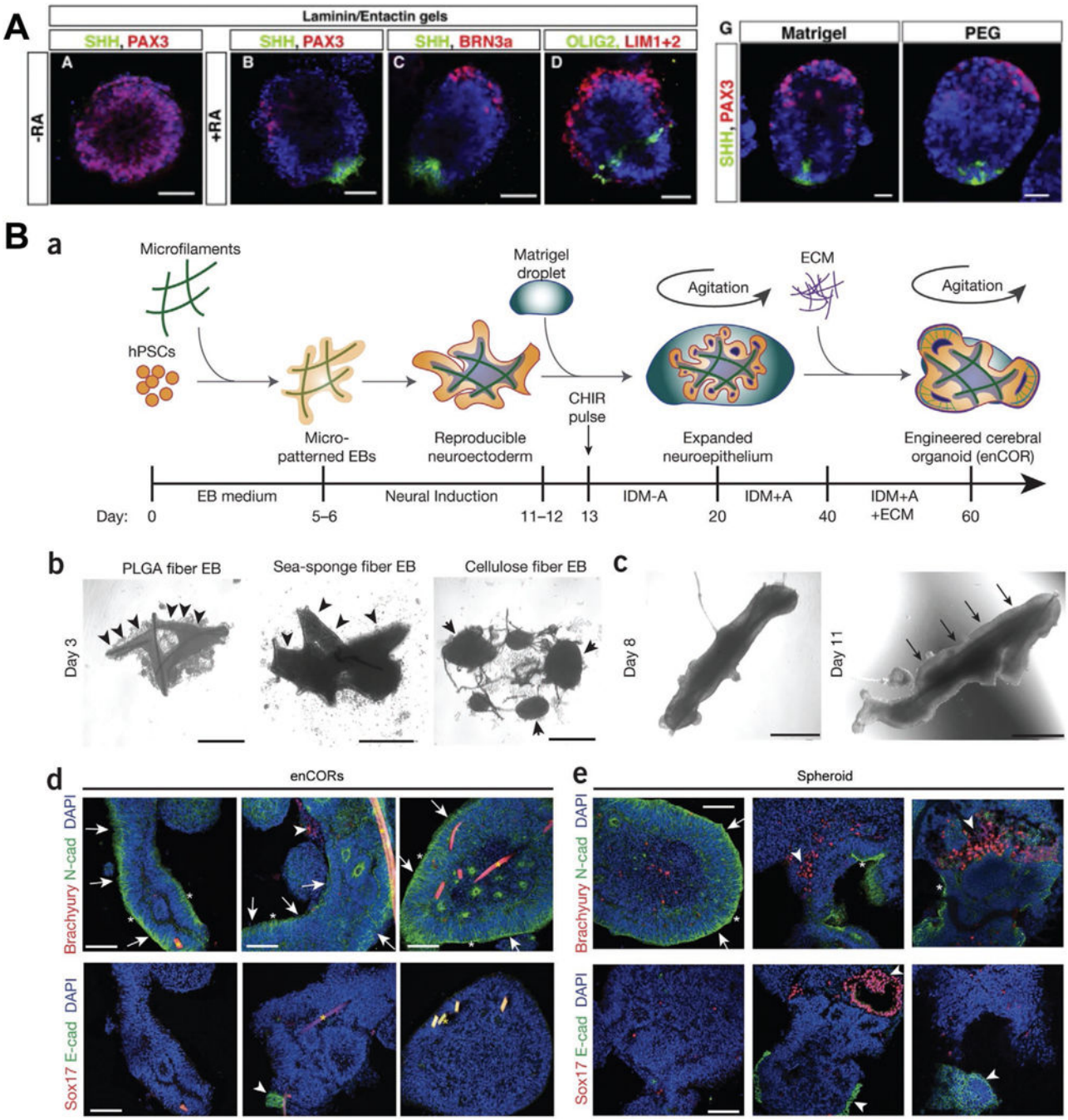

Figure 4.

Fully defined material scaffold approaches toward guided organization of organoids. A) Dorso-ventral patterning (SHH and Pax6 expression) in pluripotent stem cell derived neural tube models can be induced by defined dosages of retinoic acid using fully defined laminin and PEG scaffolds in comparison to ill-defined Matrigel scaffold; Reproduced under the terms and conditions of a Creative Commons BY-NC-ND license. Copyright 2014 The Authors, published by Elsevier. B) 60 d protocol (panel a) using a PLGA microfilament scaffold enables physical and spatial patterning for guided cerebral organoid growth (panels

b and c) and modified spatial cellular patterning and marker expression compared to an unguided spheroid patterning approach (panels d and e). Adapted with permission.[55] Copyright 2017, Springer Nature.

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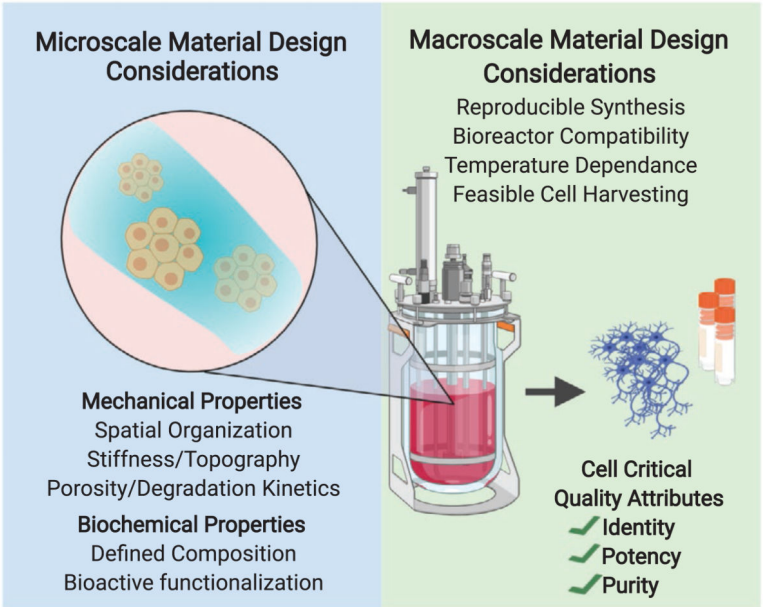

Figure 5.

Microscale and macroscale considerations for engineering materials to enhance stem cell manufacturing.

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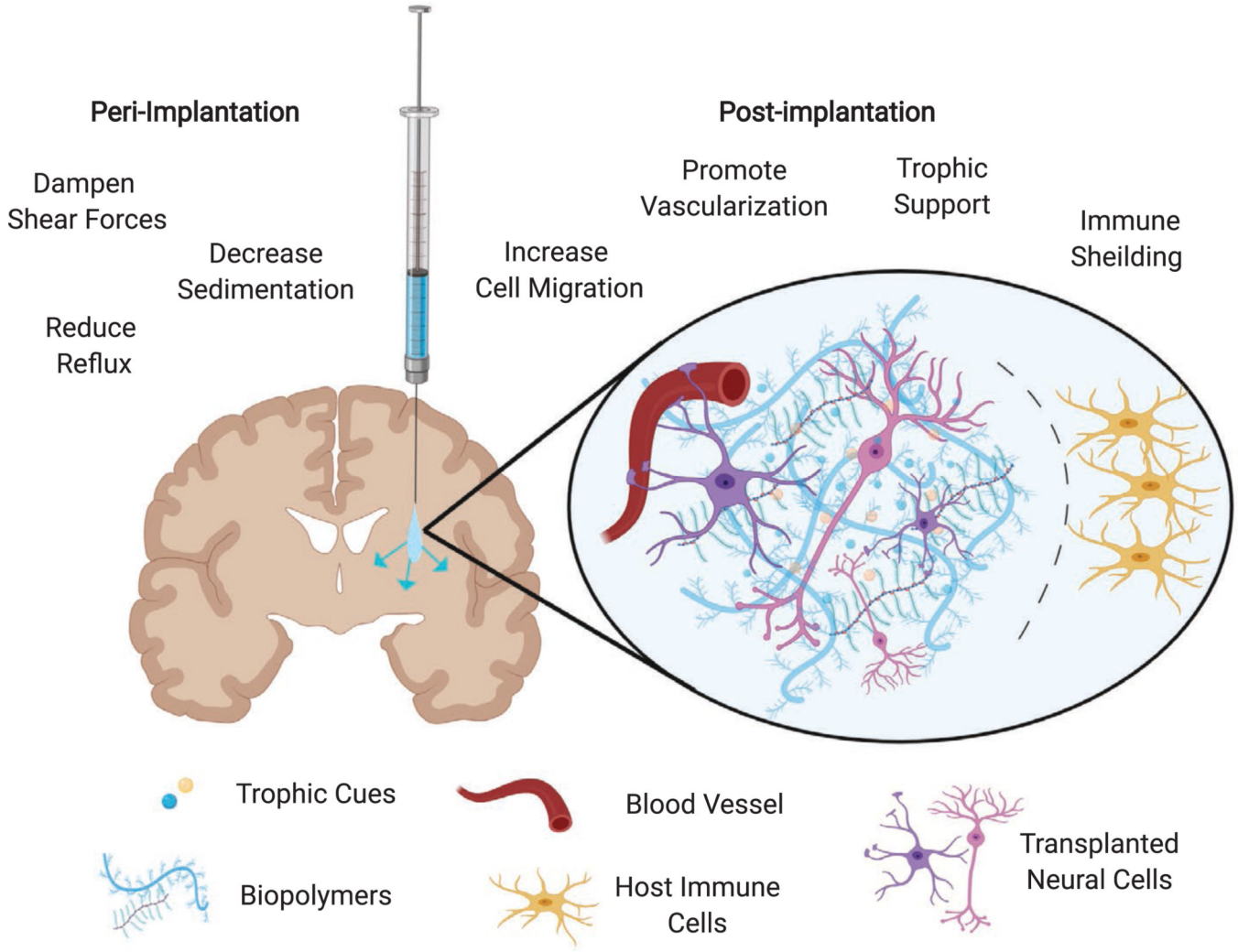

Figure 6.
Materials can be engineered to overcome problems peri- and post-implantation of neural cells into the CNS.

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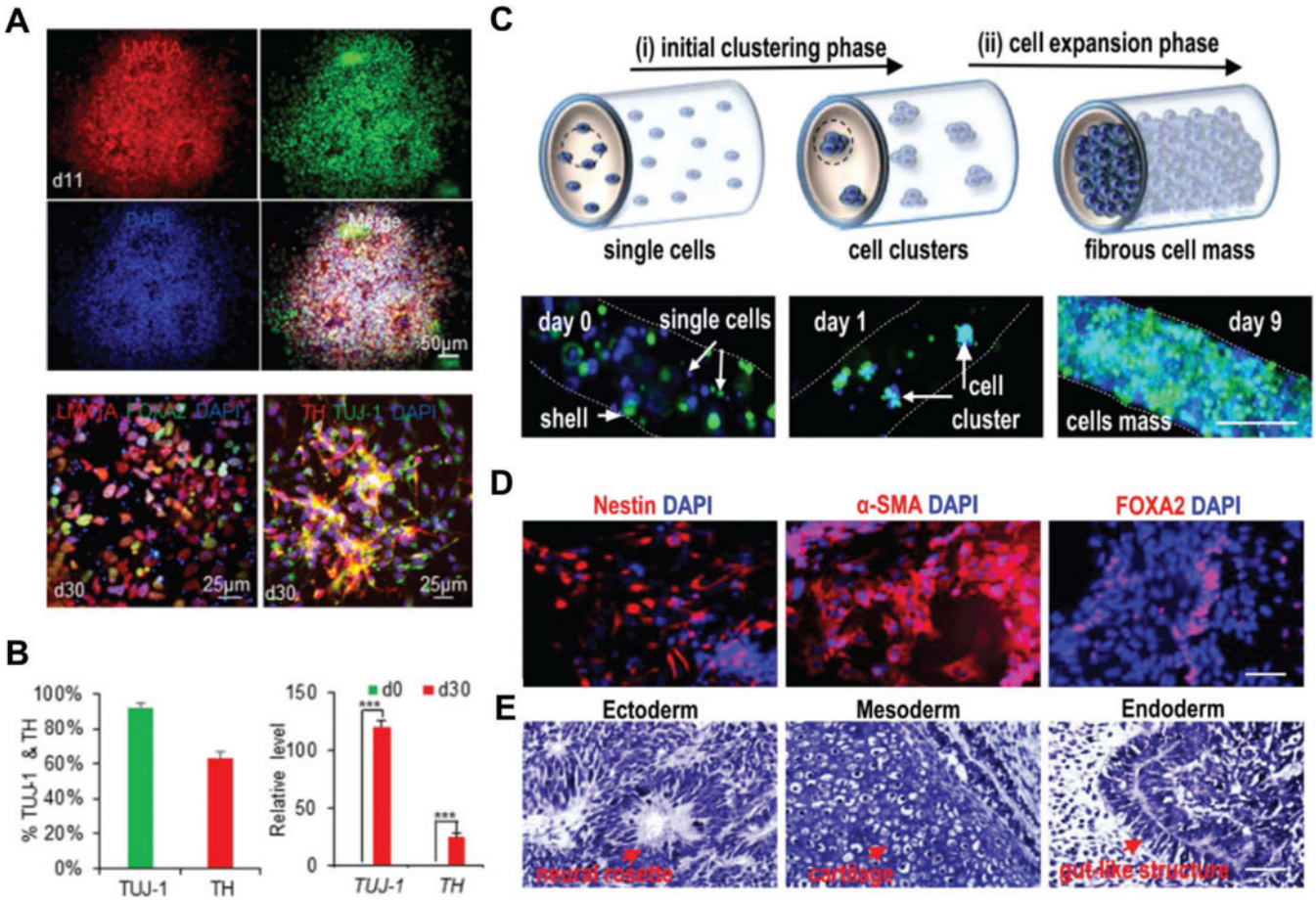

Figure 7.

Engineered materials designed to scale up stem cell manufacturing and differentiation to generate desired cell types to target neurological diseases. A) iPSCs are able to expand in 3D PNIPAAm-PEG hydrogels and B) differentiate into ventral midbrain neurons. Adapted under the terms and conditions of a CC BY license.[80] Copyright 2017, Springer Nature Limited. C) H9-hESCs expand in alginate tubes and D,E) retain their pluripotency after long-term culture in these tubes. Adapted under the terms of a CC BY license.[77] Copyright 2018, IOP Publishing.

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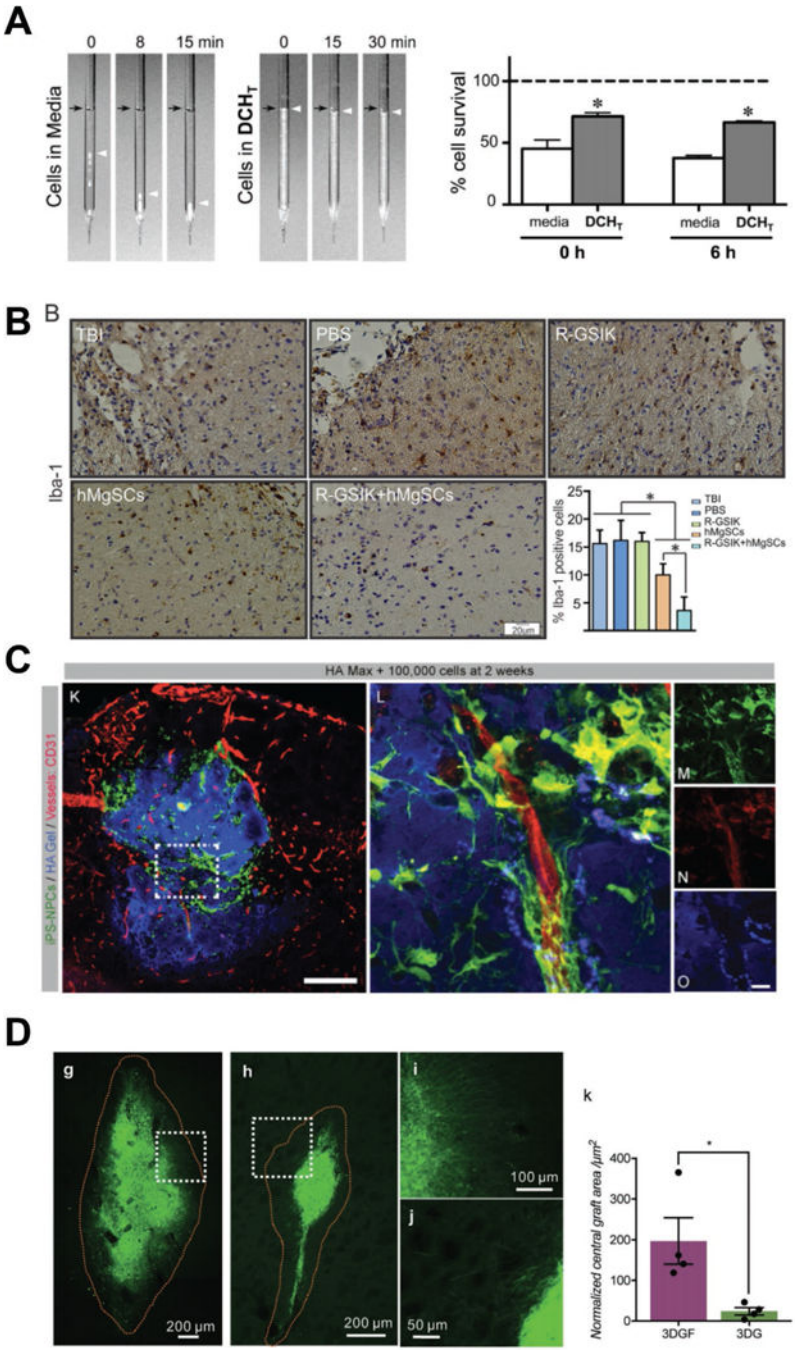

Figure 8.

Engineered materials applied toward solving several problems in neural cell transplantation in the central nervous system. A) Cells suspended in thermo-reversible DCH-T at different time points after syringe loading to visually monitor sedimentation in the syringe compared to control cells suspended in cell media. Cell survival is quantified post-injection when suspended in DCH-T and cell media; Adapted with permission from ref. [118]. Copyright 2015 American Chemical Society. B) Host microglial immune response (Iba1+ cells at graft site) and quantification comparing implantation of hMgSCs in PBS and R-GSIK; Adapted

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with permission.[138] Copyright 2019, Elsevier. C) Vascularization within lesion site measured by CD31+ expression (red) within an iPSC-NPC graft (green) implanted with hyaluronic acid (blue); Reproduced with permission.[141] Copyright 2016, Elsevier. D) HNCAM+ cells (green) implanted in a hyaluronic acid gel functionalized with growth factors (panels g and i) displayed increased graft area and cell innervation post-implantation into the striatum of a rat model of Parkinson's disease compared to cells implanted in PBS (panels h and j) and corresponding quantification of graft area (panel k); Adapted with permission.[109] Copyright 2018, Wiley-VCH.

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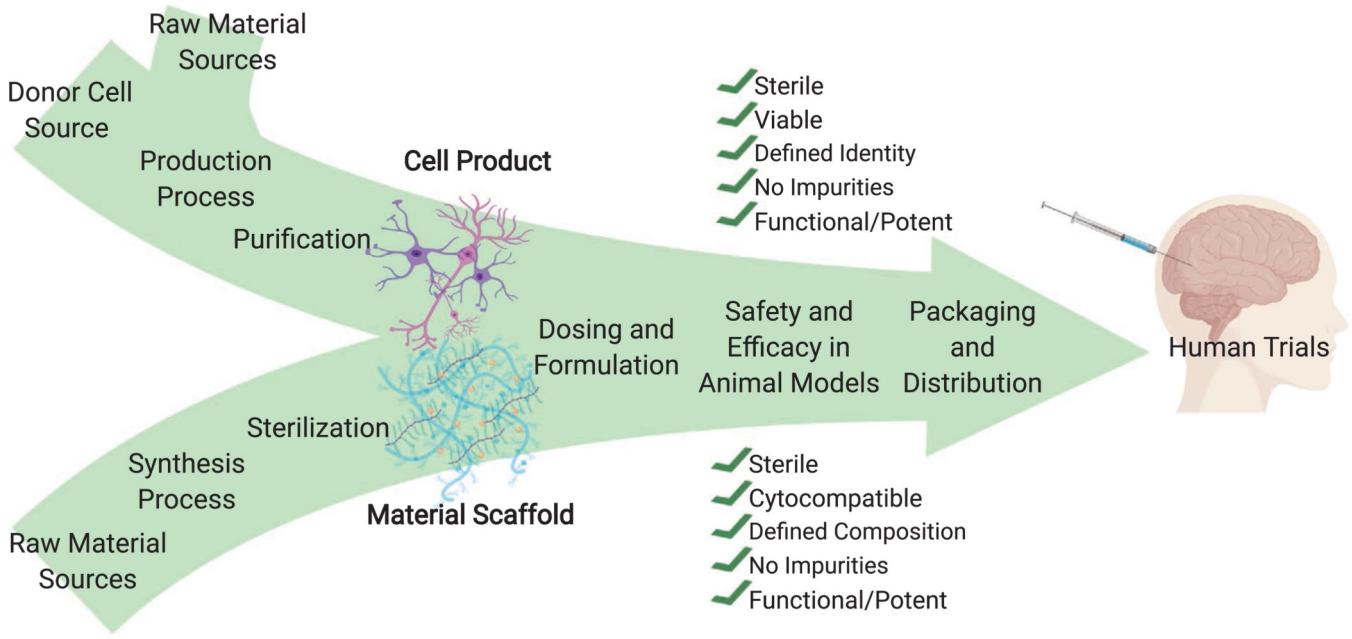

Figure 9.

Roadmap of components to consider when designing a cell therapy with material delivery vehicle.

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Table 1.

Matrigel-based self-organization in 3D models of CNS development and disease.

Application	CNS feature	Advancement made/insight gained	Refs.
Development	Cerebral cortex	Similar gene expression patterns between organoids and fetal brain	[31]
	Cerebral cortex	Differences in methylation patterns between organoids and fetal brain	[32]
	Cerebral cortex	Neuronal network formation in organoids resembles developing cortex	[33]
	Midbrain	Neuromelanin-like granule production	[178]
	Neuronal axon tracts	Presence of endogenous axon guidance cues	[179]
	Medial ganglionic eminence (MGE)	Interneuron migration	[180]
	Cerebral cortex	Vasculature and blood-brain barrier features from ETV2-expressing cells	[181]
	Brain	In vivo physiological environment	[59]
	Cortical neuroepithelium (NE)	NE grows in thickness by growth of radial glia fiber length	[37]
	Optic cup	Intrinsic self-organizing program of retinal epithelium	[182]
	Optic cup	Differences in human and mouse optic cup formation	[29]
Disease model	Dorsal and ventral forebrain	Interneuron migration in Timothy syndrome	[183]
	Cerebral cortex	Premature neural differentiation in microcephaly patients	[36]
	Alzheimer’s disease (AD)	Experimental validation of amyloid hypothesis of AD	[34]
	Dorsal forebrain	Zika virus decreases neuronal cell layer volume, microencephaly	[38–56]
	Telencephalon	Overproduction of inhibitory neurons during development of autism patients	[184]

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Table 2.

Materials for defined organization of 3D models of CNS development and disease.

Type	Material	CNS feature	Tuned properties				Insights gained	Refs.
			C	P	S	T		
Natural	Laminin/entactin	Neural tube	✓				Retinoic acid patterning is not dependant on ECM proteins or GFs in Matrigel	[52]
	Hyaluronic acid-chitosan	Forebrain in adrenoleukodystrophy	✓				Defined conditions for making organoid disease models	[185]
Synthetic	PEG	Neural tube	✓				Retinoic acid patterning is not dependant on ECM proteins or GFs in Matrigel	[52]
	PLGA microfilaments	Cerebral cortex	✓	✓	✓		Migratory patterns of neurons in developing forebrain	[55]
Synthetic hybrid	PEG (MMP sites, RGD)	Neurotoxicity	✓			✓	Can create uniform neural constructs with vascular and microglial components	[186]
	PEG (MMP sites)	Neural tube	✓	✓		✓	3D actomyosin contractility in morphogenesis	[54]

Note: C: Chemical, P: Physical, S: Spatial, T: Temporal.

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Table 3.

Materials for manufacturing pluripotent and multipotent stem cells for CNS therapies.

Cell type	Disease target	CQAs	Material used	Cell source	Culture type	Properties						Refs.
						S	Sc	D	I	B	T	
PSCs	Potential to generate all other cell types targeting several neurological diseases	Identity: Highly proliferative $OCT4^+$/$Nanog^+$/$SSEA4^+$/$Rex1^+$ cells Purity: Low numbers of $OCT4^{-}$/$Nanog^-$ cells Potency: Embryoid body formation in vitro, teratoma formation in vivo	PNIPAAm-PEG	hESCs (H1, H9), hiPSCs-MSCs and hiPSCs-Fib2	3D	✓	✓	✓	✓			[83]
			HA-PNIPAAm-PEG	hESCs (H1) and hiPSCs (TCTFs)	3D		✓	✓			✓	[116]
			PNIPAAm-PEG and alginate	hESCs (H9), hiPSCs-MSCs and hiPSCs-Fib2	3D, bioreactor		✓	✓	✓	*a)*		[77]
			poly(HPhMA-*co*-HEMA)	hESCs (HUES7) and hiPSCs (BT1)	2D	✓	✓	✓	✓			[187]
			Alginate-chitin	hiPSCs (PD-iPS5 and hFib2-iPS4)	3D		✓	✓		*a)*	✓	[155]
MSCs	Numerous neurological diseases (ALS, SCA, SCI, traumatic brain injury, MS, etc.)	Identity: Adherent cells, $CD105^+$/$CD90^+$/$CD73^+$ and $CD34^-$/$CD45^-$/$CD14^-$ Purity: Low numbers of hematopoietic stem cells ($CD45^+$/$CD34^+$) Potency: Trilineage potency, trophic effect and immunomodulation	PEG-norbornene-CRGDS	Human bone marrow	2D	✓		✓	✓		✓	[154]
			Decellularized plant tissues-RGD	Human bone marrow	2D		✓			*b)*		[156]
NSCs	Numerous neurological diseases (SCI, PD, etc.)	Identity: $Pax6^+$/$Nestin^+$/$Foxg1^+$/$Sox1/2^+$ Purity: Low numbers of mesendoderm markers ($Foxa2^+$/Gsc+/$Sox17^+$) Potency: Trilineage potency	PNIPAAm-PEG	hESCs (H9) and hiPSCs-MSCs	3D, bioreactor	✓	✓	✓	✓			[80]
			Alginate	hESCs (H9) and hiPSCs-Fib2	3D, bioreactor		✓	✓	✓	*a)*		[188]
			Polydopamine coated PS and PLGA-RGD-YIGSR-NGF-GDNF	Human fetal NSCs and hiPSC-NSCs	2D	✓	✓	✓		✓		[157]

*a)*Nondegradable in mammals, as they lack the enzyme (alginases)

*b)*Degradable by a family of hydrolytic enzymes called cellulases. However, the tightly packed and orderly structure of crystalline chains of cellulose is impervious to enzymatic degradation. For this reason, cellulosic materials show limited degradation over time.

PSCs: Pluripotent stem cells; MSCs: Mesenchymal stem cells; NSCs: Neural stem cells; CQAs: Critical quality attributes; S: Synthetic; Sc: Scalable; D: Chemically defined; I: Biologically inert; B: Biodegradable; T: Tunable stiffness; AD: Alzheimer's disease; SCA: Spinocerebellar ataxia; SCI: Spinal cord injury; MS: Multiple sclerosis; ALS: Amyotrophic lateral sclerosis; PS: Polystyrene.

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Table 4.

Materials for manufacturing stem cell-derived products for CNS therapies.

Cell type	Disease target	CQAs	Material used	Cell source	Culture type	Properties						Refs.
						S	Sc	D	I	B	T	
NPCs	Numerous neurological diseases (SCI, epilepsy, PD, etc.)	Identity: $DCX^+/Sox2^+$ Purity: Low numbers of astrocytes ($GFAP^+$), oligodendrocytes ($O4^+/Olig2^+$) and mature neurons ($Tuj1^+$) Potency: Potential to mature into $Tuj1^+/Map2^+/NeuN^+$ neurons.	HA-RGD/YIGSR/IKAV	hiPSCs	3D		✓	✓		✓		[189]
			Alginate-Chitin	hiPSCs (PD-iPS5 and hFib2-iPS4)	3D		✓	✓		*a)*	✓	[155]
mDA	PD	Identity: $En1/2^+/Otx2^+/Foxa2^+/Lmx1a^+/Nurr1^+/Pitx3^+/TH^+/Tuj1^+/Dat^+/Aadc^+/Vmat2^+/Girk2^+$ Purity: Low numbers of non-dopaminergic neurons ($Tuj1^+/TH^-$) Potency: Action potential firing, dopamine release	Self-Assembling Peptide Nanofiber	mESCs (R1) and miPSCs (TTF-1)	3D	✓	✓	✓		✓		[190]
			PNIPAAm-PEG	hESCs (H1, H9, WIBR3) and hiPSCs (TCTFs)	3D	✓	✓	✓	✓			[14,83,191]
OPCs	SCI, HD, Demyelination disorders	Identity: $PDGFaR^+/Olig2^+/NKX2.2^+/Sox10^+$ Purity: Low numbers of neurons ($Tuj1^+$), astrocytes ($GFAP^+$) and mature oligodendrocytes ($O4^+$) Potency: Proliferation and migration in vivo. Potential to mature into $O4^+/MBP^+$ myelinogenic cells.	PNIPAAm-PEG	hESCs (H9) and hiPSCs (TCTFs)	3D	✓	✓	✓	✓			[192]
MSNs	HD	Identity: $Darpp32^+/CTTIP2^+/Map2^+/Calbindin^+/GABA^+$ Purity: Low numbers of $Map2^+/GABA^-$ cells and glial ($GFAP^+$) cells Potency: Action potential firing, GABA release	PNIPAAm-PEG	hESCs (H1,H9) and hiPSCs (TCTFs)	3D	✓	✓	✓	✓			[12]
RPE	AMD, RP	Identity: $RPE65^+/BEST1^+/RLBP1^+/PMEL17^+/TYR^+/TYRP1^+/Otx2^+/CRALBP^+$ Purity: Low numbers of phase-bright cells and low side-scattering cells. Potency: Pigmentation, ROS phagocytosis, PEDF secretion	Vitronectin-mimicking oligopeptides	hESCs (H7, H9 and H14) and hiPSC (IMR90–4)	2D	✓	✓	✓				[193–195]
			RGD-alginate	hESCs (H9) and hiPSC (SB-AD3)	3D			✓				[196]
RPCs	AMD, RP, Glaucoma	Identity: $KLF4^+/Otx2^+/N\text{-}Myc^+/VSX2^+$ Purity: Low numbers of cobblestone-like, neuroepithelial-like and bipolar-like cells and phase-bright cells Potency: Differentiation into seven cell types	Vitronectin-mimicking oligopeptide poly(*ε*-caprolactone)	Human fetal retina	2D	✓	✓	✓		✓		[197]
			RGD-alginate	hESCs (H9) and hiPSC (SB-AD3)	3D			✓				[196]

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Cell type	Disease target	CQAs	Material used	Cell source	Culture type	Properties						Refs.
						S	Sc	D	I	B	T	
			Decellularized bovine retinal matrix	Human fetal retina	2D					✓		[198]
			Chitosan	miPSCs from umbilical cord fibroblasts	2D	*b)*		✓		✓	✓	[199]
			Porous PLGA	miPSCs	2D	✓		✓	✓	✓		[200]
			Polycaprolactone	Mouse and human fetal retina	2D	✓		✓		✓		[201]

a) Nondegradable in mammals, as they lack the enzyme (alginases);

b) Available in a semisynthetic formulation.

NPCs: Neural progenitor cells; mDA: Midbrain dopaminergic neurons; OPCs: Oligodendrocyte precursor cells; MSNs: Medium spiny neurons; RPE: Retinal pigment epithelium; RPC: Retinal precursor cells; CQAs: Critical quality attributes; S: Synthetic; Sc: Scalable; D: Chemically defined; I: Biologically inert; B: Biodegradable; T: Tunable stiffness; PD: Parkinson's disease; SCI: Spinal cord injury; HD: Huntington's disease; AMD: Age-related macular degeneration; RP: Retinitis pigmentosa; ROS: Photoreceptor rod outer segments; PEDF: Pigment epithelium-derived factor.

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Table 5.

Advanced materials for peri-implantation improvement of cell therapy into the CNS.

Base material	Additives	Cell type: indication	Peri-implantation			Refs.
			SF	Sed	Rfx	
DCH-T	–	NSCs: injured CNS	✓	✓		[118]
Alginate	–	MSCs, NPCs	✓			[120]
RADA16	IKVAV peptide	NSCs: cerebral neocortex loss			✓	[114]
PLGA	Collagen	Bone marrow derived MSCs		✓		[202]
HA	–	iPSC-NSCs: stroke	✓	✓	✓	[203]
HAMC	–	SCI	✓			[121]
	–	RSPCs: retinal disease		✓		[204,205]

Note: SF: Shear force reduction, Sed: Sedimentation reduction, Rfx: Reflux reduction.

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Table 6.

Advanced materials to improve post-implantation results of cell therapy in the CNS.

Base material(s)	Additives	Cell type: indication	Post-implantation					Refs.
			S/I	CM	V	TS	I	
Parylene		RPEs: AMD	✓					[106–108]
QL6 SAP	–	NPCs: SCI	✓				✓	[206]
Fibrin	Heparin, NT-3, PDGF-A	ESC-derived NPCs: SCI	✓			✓		[207]
Gelatin-PANI	–	Bone marrow stromal cells: PD	✓					[208]
DCH-T	–	NSCs: injured CNS	✓					[118]
RADA16	–	iPSC-derived mDA neurons: PD	✓					[209]
	BDNF	hUC-MSCs/astrocytes: TBI	✓	✓		✓		[210]
	IKVAV peptide	NSCs: cerebral neocortex loss	✓			✓		[114]
	IKVAV peptide	Meningioma stem like cells: TBI	✓			✓	✓	[138]
PCL	Immobilized GDNF	Primary cortical NSCs: brain parenchyma	✓	✓		✓	✓	[159]
PLA-xyloglucan	GDNF	mDA neural progenitors: PD	✓			✓		[211]
PLGA	VEGF	NSCs: stroke	✓		✓			[212]
Collagen	–	GDNF-MSCs: striatal parenchyma					✓	[213]
	–	MSCs: TBI	✓					[214]
Gelatin-methacrylate	–	iPSC-derived NSCs: SCI	✓				✓	[146]
PuraMatrix	–	NSCs, ESCs: cortical contusion injury	✓		✓		✓	[215]
	–	NSCs: TBI	✓					[216]
	–	hNSCs: TBI	✓				✓	[217]
Crosslinked methylcellulose	Chondroitinase ABC	NPCs: SCI	✓				✓	[218]
Hyaluronic acid	–	iPSC-NSCs: stroke					✓	[203]
	–	ReNcells, GRPs: intracerebral injection	✓					[219]
	Heparin	ESC-derived NPCs: stroke	✓				✓	[220]
	RGD, heparin	hESC-derived mDA neurons: intrastriatal injection	✓					[191]
	MMP sites; IKVAV, YIGSR, RGD peptides; BDNF, BMP-4	iPSC-NSCs: stroke	✓		✓	✓		[148]
HA-alginate	–	hUC-MSCs: TBI	✓					[221]
HA-DBCO-PEG	Heparin, Ephrin B2, HGF, GDNF	hESC-derived mDA neurons: PD	✓	✓		✓		[109]
HA-methylcellulose	–	RSPCs: retinal disease	✓	✓				[204,205]

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Base material(s)	Additives	Cell type: indication	Post-implantation					Refs.
			S/I	CM	V	TS	I	
	Chondoitinase ABC	iPSC-derived NESCs: SCI	✓				✓	[222]
	PDGF-A	Adult NSCs:SCI	✓			✓	✓	[223]
	RGD, PDGF-A	iPSC-derived OPCs: SCI	✓			✓		[224]

Note: S/I: Survival/integration, CM: Cell migration, V: Vasculature, TS: Trophic support, I: Immune shielding.